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SHORT SUMMARY

During the de-etiolation process, light triggers the accumulation of the core microprocessor components and pri-miRNAs, but not miRNAs. Light-induced suppression of DCL1 activity and SDN1-induction modulate this phenomenon, called miRNA-biogenetic inconsistency, and that is essential for the survival of de-etiolated seedlings after long-term skotomorphogenesis.

ABSTRACT

The shift of dark-grown seedlings into light causes enormous transcriptome changes followed by a dramatic developmental transition. Here, we show that miRNA biogenesis also undergoes regulatory changes during de-etiolation. Etiolated seedlings maintain low levels of primary-miRNAs (pri-miRNAs) and miRNA processing core proteins, such as Dicer-like 1 (DCL1), SERRATE (SE) and HYPONASTIC LEAVES 1 (HYL1), whereas during de-etiolation, both pri-miRNAs and the processing components accumulated to high levels. However, most miRNA levels did not notably increase in response to light. To reconcile this inconsistency, we demonstrate that an unknown suppressor decreases miRNA-processing activity and light-induced SMALL RNA DEGRADING NUCLEASE 1 (SDN1) shortens the half-life of several miRNAs in de-etiolated seedlings. Taken together, we suggest a novel mechanism, miRNA-biogenetic inconsistency, which accounts for the intricacy of miRNA biogenesis during de-etiolation. This mechanism is essential for the survival of de-etiolated seedlings after long-term skotomorphogenesis and their optimal adaptation to ever-changing light conditions.

INTRODUCTION

Light regulates multiple developmental processes in plants, including seed germination, seedling de-etiolation, shade avoidance, phototropism, flowering time, photoperiod, and the circadian rhythm (Casal, 2012; Deng and Quail, 1999; Li et al., 2011; Lin, 2000; Lin, 2002; Neff et al., 2000; Roux et al., 1994). Among the light-induced developmental processes, seedling de-etiolation is the most remarkable. Photoreceptors perceive light signals and, via interacting transcription factors, rapidly change the expression of numerous downstream target genes. Such orchestration of photoreceptor signaling induces dramatic transcriptome shifts, characteristic of de-etiolation (Labuz et al., 2012; Nelson et al., 2000; Reed et al., 1994; Tepperman et al., 2001; Wang et al., 2016; Yang et al., 2000). Light-dependent signaling governed by photoreceptors alters the expression of many transcription factors and induces transcriptional cascades, thereby rapidly changing the expression profiles of numerous down-stream genes (Duek and Fankhauser, 2003; Hong et al., 2008; Jiao et al., 2003; Kang et al., 2005; Tepperman et al., 2001).

MicroRNAs (miRNAs) are small non-coding RNAs that function in mRNA

degradation or translational repression (Chen, 2005; Huntzinger and Izaurralde, 2011; Pillai, 2005). The processing of plant miRNAs, from primary miRNA transcripts (pri-miRNA) requires the type III RNAse, DICER-LIKE1 (DCL1), and two RNA-binding proteins—SERRATE (SE), and HYPONASTIC LEAVES1 (HYL1), known as the core microprocessor (Kurihara et al., 2006; Kurihara and Watanabe, 2004; Yang et al., 2006; Yang et al., 2010). Mature miRNAs are methylated by HUA ENHANCER 1 (HEN1) for stabilization (Li et al., 2005), and known to be exported to the cytoplasm by HASTY, and loaded onto ARGONAUTE 1 (AGO1) (Bartel, 2004; Park et al., 2005). However, a recent study showed that miRNA translocation and functionality entail the nuclear-cytoplasmic shuttling of AGO1 (Bologna et al., 2018). Furthermore, many recent discoveries witnessed the diverse functions of AGO1 (Ma and Zhang, 2018). Core microprocessor activity is regulated negatively or positively by many proteins such as C-TERMINAL DOMAIN PHOSPHATASE-LIKE 1 (CPL1), SNF1-RELATED PROTEIN KINASE 2 (SnRK2), NOT2, KARYOPHERIN ENABLING THE TRANSPORT OF THE CYTOPLASMIC HYL1 (KETCH1), RECEPTOR FOR ACTIVATED C KINASE 1 (RACK1), PROTEIN PHOSPHATASE 4 (PP4), SUPRESSOR OF MEK1 (SMEK1), RNA DEBRANCHING ENZYME 1 (DBR1), TOUGH (TGH), DAWDLE (DDL), and CHROMATIN REMODELING FACTOR 2 (CHR2) (Li et al., 2016; Manavella et al., 2012; Ren et al., 2012; Speth et al., 2013; Su et al., 2017; Wang et al., 2013; Wang et al., 2018; Yan et al., 2017; Yu et al., 2008; Zhang et al., 2017). Many components such as STABILIZED 1 (STA1), INCREASED LEVEL OF POLYPLOIDY1-1D (ILP1) and NTC-RELATED PROTEIN 1 (NTR1) are also involved in miRNA biogenesis by removing introns of intron-harboring pri-miRNAs, promoting, and facilitating transcriptional elongation of *MIRNA* (*MIR*) genes, respectively (Ben Chaabane et al., 2013; Wang et al., 2019a). Furthermore, MOS4-ASSOCIATED COMPLEX (MAC) plays roles in modulating miRNA levels through adjusting pri-miRNA transcription, processing, and stability (Jia et al., 2017).

Light mediated changes in *MIRNA* gene expression has been observed in different plant species (Li et al., 2014; Mancini et al., 2016; Qiao et al., 2017; Shikata et al., 2014; Sun et al., 2015; Xie et al., 2017; Xu et al., 2017; Zhang et al., 2011). In Arabidopsis, white-light pulse treatment of etiolated seedlings reportedly increased the expression of *MIR157*, *MIR163*, and *MIR398,* but reduced that of *MIR408*, *MIR822*, and *MIR834* (Mancini et al., 2016). Red-light altered the expression of *MIR163*, *MIR156c*, *MIR157c*, *MIR169l*, and *MIR824a* (Shikata et al., 2014*)*. A genome-wide mapping study revealed that ELONGATED HYPOCOTYL 5 (HY5) recognizes the promoter regions of at least eight *MIRNA* genes (Zhang et al., 2011).

PHYTOCHROME INTERACTING FACTORS (PIFs) bind to the promoters of five *MIR156* genes to suppress their expression, showing that light can directly control *MIRNA* gene expression (Xie et al., 2017). Furthermore, light can affect the expression and localization of HYL1; CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) protects HYL1 from an unidentified cytoplasmic protease in a light-dependent manner (Cho et al., 2014). Moreover, the nuclear localization of HYL1 is maintained by phosphorylation under darkness (Achkar et al., 2018). Light-induced expression of *HEN1* is mediated by *HY5* and its homolog *HYH* (Tsai et al., 2014). However, it is increasingly evident that not only does light modulate miRNA processing, but light-induced development itself is affected by miRNA-biogenetic components and miRNAs. For example, deficiency of *DCL1*, *AGO1, HYL1*, *HASTY*, or *HEN1* results in shorter hypocotyls than those in wild-type seedlings under dark and light conditions (Lu and Fedoroff, 2000; Sorin et al., 2005; Sun et al., 2018; Tsai et al., 2014). The light-responsive miRNAs, miR157 and miR319 promote the degradation of *HY5* and *TCP24* transcripts, key positive and negative transcription factors of photomorphogenesis, respectively (Tsai et al., 2014). Compared to the wild-type, *mir390b* and *mir160b* mutants display long hypocotyl phenotypes, whereas the *mir167b* and *mir848* mutants have short hypocotyl phenotypes, indicating either a positive or negative role for specific miRNAs in photomorphogenesis (Sun et al., 2018).

However, the detailed crosstalk between light signaling and miRNA biogenesis during the de-etiolation process remains elusive. In the present study, we show how light adjusts the functionality of microprocessor components and subsequently the miRNAome profiles to drive a light-responsive transcriptome shift, and thereby the survivability of de-etiolated seedlings.

RESULTS

Light induces accumulation of core microprocessor components

We previously showed that HYL1 is a short-lived protein and is stabilized by light (Cho et al., 2014). This prompted us to test whether other core microprocessor proteins such as DCL1 and SE are also regulated by light. Therefore, five-day-old dark-grown (hereafter referred to as 5D) seedlings were transferred to continuous white light. After three days, the abundance of DCL1, SE, and HYL1 was determined and found to be low in etiolated seedlings. Upon exposure to white light, all tested proteins accumulated to higher levels each day (Figure

1). Plants detect and respond to specific wavelengths of light such as blue, red and far-red. So, we also monitored the levels of DCL1, SE and HYL1 proteins in seedlings exposed to monochromatic irradiation. Levels of DCL1, SE and HYL1 increased after all three light treatments as compared to de-etiolated seedlings. However, the degree of induction was consistently higher in response to blue and red light than in response to the far-red light treatment (Figures 1A-1C). DCL1 protein abundance rapidly decreased in four-day-old dark-grown (hereafter referred to as 4D) seedlings irradiated with red light for 3-5 h (Sun et al., 2018). This result contradicts our findings because we observed that all the three proteins accumulated in response to each monochromatic light treatment (Figure 1D). To further validate the light-induced accumulation of DCL1, we created transgenic plants expressing DCL1-6myc protein under the control of the constitutive *35S* promoter. DCL1-6myc protein accumulated to high levels under white light and monochromatic irradiation (Figures S1A-S1C). Moreover, we observed a similar increase in DCL1-6myc levels after a few hours of irradiation of 4D seedlings (Figure S1D). These data support our conclusion that light increases DCL1 as well as SE and HYL1 protein levels (Figures S1A-S1C). In addition, we tested the effect of light to dark transition using two-week-old plantlets grown under continuous white light. When the plantlets were transferred to darkness for 12 h, the levels of DCL1 and HYL1 decreased notably, while that of SE remained unaltered (Figure 1E). This finding is consistent with our previous report (Cho et al., 2014) and implied that the SE level is differentially regulated in plantlets grown under constant light condition. To elucidate whether the expression of microprocessor components was modulated at the level of mRNA or protein, we examined the levels of *DCL1*, *SE*, and *HYL1* transcripts in etiolated and de-etiolated seedlings using quantitative reverse transcription polymerase chain reaction (qRT-PCR). We found that *DCL1*, *SE*, and *HYL1* transcripts were unaltered after light exposure for one day. However, all the tested transcripts were up-regulated by at least 2-fold in the extended light treatments over two days (Figure 1F). We speculated that light affected the whole transcriptome including many well-known internal reference genes. Thus, we selected *UBQ10* as a reference gene having the least fluctuation to determine fold-differences in the expression of target genes (Figures S2A-S2C; see methods). Next, we treated 4D seedlings with carbobenzoxy-Leu-Leu-leucinal (MG132) or protease inhibitor cocktail (PIs) to examine the role of possible proteolytic degradation of the microprocessor components. We detected accumulation of DCL1, SE, and HYL1 in response to these chemical treatments, indicating the degradation of microprocessor components in darkness (Figure 1G).

Furthermore, we applied these chemicals to 4D Col-0/*35S:DCL1-6Myc* transgenic seedlings, resulting in identical patterns of protein accumulation (Figure S1E). Based on these results, we speculated that DCL1 could be degraded by protease or proteases (Cho et al., 2014). In addition, we also found that the core microprocessor proteins are synthesized *de novo* after germination (Figure S1F). In darkness, the levels of the components gradually increased for 4 days after germination and dropped after 5 days (Figure S1F). Conclusively, our results indicated that irradiation increases the abundance of these microprocessor components via altered transcriptional regulation and modulation of protein half-life.

Light signaling pathways meditate the accumulation of microprocessor proteins

Photoreceptors are key light sensing molecules that mediate light signaling in plants. Therefore, we investigated which photoreceptors are involved in the light-induced accumulation of microprocessor proteins. We grew *phyA*/*phyB* and *cry1*/*cry2* double mutant plants in darkness for 5 days and shifted them to white light for 1-3 days or alternatively for 17-days under continuous white light (CL). Under white-light irradiation, DCL1, SE and HYL1 accumulated in the tested mutants (Figures 2A, 2B, S2D and S2E). Next, we tested whether the accumulation of the microprocessor proteins occurs under monochromatic irradiation with different light sources. DCL1, SE, and HYL1 did not accumulate to high levels in *phyA*/*phyB* mutants under red or far-red light (Figures 2C and 2D). Likewise, the core microprocessor components did not accumulate notably in the *cry1*/*cry2* mutant under blue light (Figure 2E). We also germinated *phyA/phyB* mutants under red or far-red light and the *cry1/cry2* mutant under blue light. The four-day irradiation with each light type did not result in the accumulation of the microprocessor components in those mutant backgrounds (Figure 2F). These observations suggest that the light-induced accumulation of core microprocessor components is modulated by phytochromes A and B under red, and far-red, whereas cryptochromes 1 and 2 governed the photoreceptor-mediated pathways under blue light. These show that monochromatic light treatments - blue, red, and far-red light - can independently trigger the accumulation of the microprocessor components through these photoreceptors.

Primary-miRNAs are highly up-regulated during de-etiolation process

Even though up- and down-regulation of *MIRNA* genes in response to various environmental changes have been reported (Li and Zhang, 2016; May et al., 2013; Sunkar et al., 2012), the expression profiles of pri-miRNAs in response to light are not entirely clear. We investigated the expression profiles of known pri-miRNAs in etiolated seedlings that were irradiated with white light. Using qRT-PCR, we found that the transcript levels of these pri-miRNAs increased at least 3-fold in de-etiolated seedlings compared to that in the five-day-old etiolated seedlings grown in the dark. For instance, *pri-miR163* and *pri-miR159* transcripts were dramatically up-regulated over 400-fold and 40-fold in de-etiolated seedlings, respectively. *Pri-miR157*, *pri-miR160*, *pri-miR165*, *pri-miR390* transcripts increased over 15-fold in response to light. In the case of *pri-miR164*, *pri-miR166*, *pri-miR168*, *pri-miR171*, *pri-miR172*, *pri-miR319*, *pri-miR393*, *pri-miR396* and *pri-miR403 t*ranscript levels increased 3 to 10-fold (Figure 3A). These pri-miRNAs showed light-responsive accumulation patterns. Therefore, in line with the light-responsive increment of microprocessor components, we hypothesized that light may accelerate miRNA production during photomorphogenesis.

MiRNAome selectively shifts during the early stage of de-etiolation

To test the hypothesis that the miRNAome shift occurs during de-etiolation, we investigated the steady-state-levels of mature miRNAs in de-etiolated seedlings and compared them to that in the etiolated seedlings. We performed two independent sets of miRNA sequencing experiments on samples of five-day-old etiolated (5D) seedlings and of 5D seedlings that were irradiated with white light for one day (hereafter referred to as 5D+1L) or for three days (hereafter referred to as 5D+3L). We found that the median expression level of miRNAs was slightly altered in 5D+1L seedlings (change-ratio: $\log_2\Delta$TPTM value of 0.13 for set-1; 0.18 for set-2) and in 5D+3L seedlings (the change ratio of -0.01 for set-1; 0.07 for set-2) (Figure S3A). Of the 151 miRNAs that had a total expression of at least twenty transcripts per 10 million (TPTM), nearly two-thirds of the miRNAs had higher expression in 5D+1L seedlings (60% for set-1; 63% for set-2) and in 5D+3L light-treated seedlings (60% for set-1; 62% for set-2) (Figures S3B and S3C). However, we noted that most of the miRNAs whose expression increased in all the light-treated seedlings (43%~46%) had increased to less than 0.5 (change-ratio) as compared to that in the 5D

196 seedlings. Likewise, most of the reduced miRNAs (32%~34%) decreased between 0 and 0.5
197 (change-ratio) (Figure S3C). When we filtered the miRNAs for those with read-counts over
198 100 reads, only 25 miRNAs (17%) from set-1 and 26 miRNAs (17%) from set-2 increased
199 above 0.5 in both, 5D+1L and 5D+3L seedlings (Figure 3B). Among these, miR157 (3.9 for
200 both set-1 and set-2 in 5D+1L seedlings; 4.8 for both set-1 and set-2 in 5D+3L seedlings)
201 and miR163 (7.4 for both set-1 and set-2 in 5D+1L seedlings; 7.7 for both set-1 and set-2 in
202 5D+3L seedlings) were most remarkably up-regulated by light. Besides, only 8 miRNAs
203 (5%) for set-1 and 7 miRNAs (5%) for set-2 decreased below -0.5, and miR406 (-1.2 for
204 both sets in 5D+1L seedlings; -2.4 for both sets in 5D+3L seedlings) and miR8176 (-3.1 for
205 set-1 and -1.9 for set-2 in 5D+1L seedlings; -1.0 for both set-1 and set-2 in 5D+3L seedlings)
206 decreased after both light treatments (Figure 3C). In addition to these miRNAs, 22 miRNAs
207 from both, sets-1 and 2, were specifically up-regulated ($\log_2\Delta$TPTM > 0.5), while 5 miRNAs
208 from set-1 and 6 miRNAs from set-2 were notably down-regulated ($\log_2\Delta$TPTM < 0.5) in
209 5D+3L seedlings. In particular, miR397, miR8175, miR399, and miR408 notably increased
210 in 5D+3L seedlings. Conversely, miR845 and miR858 specifically decreased in 5D+3L
211 seedlings (Figure S4A). Given that we analyzed miRNAs based on a high change-ratio in
212 response to light, several miRNAs without notable changes ($\log_2\Delta$TPTM < 0.5 or >-0.5)
213 were excluded in the analysis, regardless of their abundance. Furthermore, many miRNAs
214 shown in Figure 3B and 3C are less-defined in their functions. Therefore, we further
215 analyzed well-defined and abundant miRNAs with read-counts over 20,000. We found that
216 miR162, miR173, and miR408 significantly increased, while miR156, miR159, miR165,
217 and miR396 consistently decreased with the light treatments (Figure S4B). We monitored
218 the change in number of reads (ΔTPRM) to confirm the actual number of miRNAs.
219 Interestingly, we found that miR158 (ΔTPRM = ~400,000 to ~800,000 reads) had a greater
220 increase than did miR163 (ΔTPRM = ~100,000 reads) and miR157 (ΔTPRM = ~100,000
221 reads) (Figure S4C). This result implied that miR158 could be an important light-responsive
222 miRNA even though its change-ratio is less than 0.5. In the same context, we found that the
223 greatest decrease in ΔTPRM occurred for miR156 and miR159 (Figure S4D). The duration
224 of the light exposure differently regulated several miRNAs. For instance, miR166, miR319,
225 and miR168 slightly increased in 5D+1L seedlings and then reduced in 5D+3L seedlings. In
226 contrast, miR167, miR161, and miR403 reduced in 5D+1L seedlings, and then increased in
227 5D+3L seedlings (Figure S4E). Using northern blot analysis, we further confirmed the light-

responsiveness of selected miRNAs. Consistent with the sequencing results, miR156 and miR159 decreased, miR166 fluctuated, and miR157 increased (Figure 3D). Lastly, we performed miRNA sequencing analysis using the third set of samples, which were independently treated with white light on 5D seedlings for a day (5D+1L). We obtained a similar expression pattern as presented in Figure S3C (Figure S4F). Taken together, we found that most miRNAs are not notably altered during skoto- to photo-morphogenic developmental transition and our hypothesis mentioned above is improbable.

Primary miRNAs and the core microprocessor components further accumulated in response to prolonged light treatment

The miRNAome shift in the continuous light-grown plants compared to that in the etiolated seedlings prompted us to test the levels of miRNA precursors and microprocessor proteins under prolonged irradiation after the skoto- to photo-morphogenic transition. Therefore, we investigated the miRNA biogenesis in de-etiolated seedlings exposed to prolonged light for 12 days (5D+12L) and in CL seedlings. To test the light-induced accumulation of pri-miRNAs and their correlation to mature miRNAs, we performed northern blot analyses and tested the expression levels of *pri-miR156a-j*, *pri-miR159a-c*, *pri-miR163*, *pri-miR166a-f*, and *pri-miR319a-c* transcripts by using qRT-PCR. We observed that all the tested pri-miRNAs increased after 3-12 days of WL irradiation in 5D seedlings. The levels of pri-miRNAs in 5D+12L seedlings were similar to those observed in CL plantlets (Figure 4A-4E). Despite the dramatic increase in miRNA precursors, the amount of mature miR156, miR159, and miR166 decreased and the levels of miR163 and miR319 were not notably altered after 9-12 days of irradiation or in the CL plantlets as compared to that observed in 5D+1L seedlings (Figure 4F). These results corroborate the miRNAome analysis (Figure 4G and S6). We further tested whether the expression of microprocessor components was altered by such prolonged light conditions. Transcripts of the components were not further increased after two or three days of light treatments (Figure 4H). Moreover, the levels of all the microprocessor proteins gradually increased when the light treatments were extended (Figure 4I). The reduced or unaltered miRNAs in the de-etiolated seedlings and the light-grown plantlets were not easy to comprehend considering the levels of microprocessor and pri-miRNAs amassed: hereafter, this phenomenon is referred to as the miRNA-biogenetic inconsistency.

Light lowers pri-miRNA processing activity of the microprocessor

We hypothesized that the miRNA-biogenetic inconsistency might be caused by unknown negative regulatory pathways during photomorphogenesis, light adaptation, and photomorphogenic growth under constant light. To verify the existence of the unknown negative regulatory pathways, we formulated three possible scenarios: 1) the enzyme activity of the core microprocessor could be different between etiolated and de-etiolated seedlings, 2) the presence of a light-induced repressor of the core microprocessor, or 3) differential regulation of miRNA turnover. To test the first scenario, we investigated the enzyme activity of the core microprocessor using Col-0/*35S:DCL1-6Myc* transgenic seedlings. First, we purified the core microprocessor complex using α-myc antibody from 5D and 5D+1L transgenic seedlings. Then, the immunoprecipitated microprocessor complex was incubated with ^{32}α-UTP isotope incorporated pri-miR166c as a substrate (Figure 5A). We observed that four intermediate fragments and a miRNA-like fragment only in the reaction using immunoprecipitates from Col-0/*35S:DCL1-6Myc* transgenic seedlings (Figure 5B). Next, to confirm whether the observed lower band having the size of a miRNA is really miR166, we used non-labeled pri-miR166c substrate for the enzymatic assay and performed PAGE before subsequent blotting and hybridization with ^{32}γ-ATP labeled miR166-specific probe or non-miR166 probe as a negative control. We detected a specific band which has the same size as the synthetic miR166 and was recognized by only by the radioactively labelled miR166-specific probe (Figure 5C). Based on these control experiments, we further examined the DCL1 enzyme activity between dark-grown 5D and 5D+1L de-etiolated seedlings. We observed that the cleavage activity of the purified microprocessor in 5D+1L seedlings was approximately similar to that of 5D seedlings, even though the protein level of microprocessor was over 10-fold higher in 5D+1L seedlings (Figure 5D). Thus, we equilibrated the amount of DCL1-6myc, to compare the unit activity between the two samples, and found that the processing activity was much higher in 5D seedlings (Figure 5E). As shown in Figure 3, *pri-miR166a* and *pri-miR390a* transcripts accumulation was highly induced by light (about 4.5- and 25-fold, respectively). To search for the reason of this phenomenon, we tested the promoter activity of *MIR166a* and *MIR390a* under these circumstances. Although the expression levels of the examined reporter genes were not influenced by light (Figure S7), we cannot rule out that other

MIR genes can be specifically light-regulated (Chung et al., 2016). This result suggests that not gene expression changes but decreased microprocessor activity triggered by light could lead to the accumulation of many pri-miRNAs.

To further examine this scenario, we adopted the micro-Protein-DCL1 (miP-DCL1) system that ectopically expresses DCL1-PAZ domain, producing more miRNAs, possibly by titrating a yet unidentified potential negative regulator (Dolde et al., 2018) (Figure 6A). Using 17-day-old light-grown Col-0/*35S:miP-DCL1* transgenic plants, we showed the increased amounts of miR156, miR159 and miR319 and the notable reduction of *pri-miRNA* transcripts as compared to wild-type plants, thus confirming the positive role of miP-DCL1 in miRNA biogenesis (Dolde et al., 2018)(Figures 6B and 6C). Next, we tested whether the production of miRNAs is influenced by overexpressing miP-DCL1 during the de-etiolation process. We found that the light-reduced miRNAs - miR156, miR159, miR166, and miR319 - accumulated to higher levels in miP-DCL1 seedlings compared with wild-type plantlets after 1-3 days of light treatments (Figures 6D - 6F) indicating miP-DCL1 impact on miRNA levels during de-etiolation. Likewise, the production of light-increased miRNAs such as miR157 and miR163 (Figures 3B and S5C) were more distinctively elevated in miP-DCL1 seedlings than in wild-type seedlings (Figures 6D-6F). This result though intriguing, implied that this unknown negative regulator also suppressed the production of light-induced miRNAs to a certain degree. Considering the similar levels of tested miRNAs in the 5D seedlings of miP-DCL1 and wild-type, the light-driven miRNA-biogenetic inconsistency could be caused by reduced microprocessor activity. With these results, we speculated that the first and second scenarios are plausible to account for light-induced miRNA-biogenetic inconsistency. Furthermore, we found that the up-regulated miRNA levels in Col-0/*35S:miP-DCL1* transgenic plants led to proportional defects in cotyledon opening (Figure 6G). In sugar-free medium, 5D+1L seedlings of miP-DCL1 showed a delay in cotyledon opening (25-30%), while 5D+1L seedlings of Col-0 had fully-opened cotyledons (100%). Moreover, when 6D seedlings of miP-DCL1 - in which the endosperm could be depleted - were irradiated with white light for 5 days, only 50-55% of the seedlings survived via photomorphogenesis, while 90% of 6D+5L seedlings of Col-0 survived (Figures 6G and 6H). Light-grown seedlings of miP-DCL1 had defects in leaf development, flowering time, and growth (Figures 6I and S8). These results indicated the down-regulation of microprocessor activity by light, which is important for normal photomorphogenesis and development.

The half-life of miRNAs is differentially regulated during de-etiolation

While it was not possible to rule out the activity of miRNA-decaying RNases such as SDN1, SDN2, and SDN3 that can modulate the half-life of miRNAs, possibly leading to miRNA-biogenetic inconsistency, we found that *SDN1* expression is highly up-regulated during photomorphogenesis (Figures 7A and 7B). RNA polymerase II is responsible for the *MIRNA* gene expression, and α-amanitin is a specific inhibitor (Rajjou et al., 2004). Thus, we monitored the half-life of miRNA by treating 5D+2L seedlings and 5D seedlings with α-amanitin for 6 hours (Figure 7C). Under these experimental conditions, several miRNAs such as miR156, miR157, miR158, miR159, miR160, miR169, and miR319 decayed, while miR163, miR166, miR167, miR168, miR171, miR390, and miR393 were either unaltered or increased in the 5D+2L seedlings. By contrast, only miR157 and miR158 notably decreased in the 5D seedlings, implying that the third scenario is also applicable. This result indicated that miRNAs could have different turnover rates during the de-etiolation process. The outcome of α-amanitin-chase assay was in concordance with the expression profiles of several miRNAs. For instance, miR156 and miR159 rapidly decayed; therefore, their levels notably decreased, whereas miR163 was stable; accordingly, its levels increased with light irradiation treatments (Figure 4G). Even though several miRNAs have a short half-life, their amounts did not decrease; instead, miR157 (16-fold) and miR158 (1-fold) increased, while miR160 and miR319 were less altered by light exposure (Figures 3B, 7C, S4E and 4G). To account for the different expression levels of these rapidly-decaying miRNAs, we hypothesized the varied abundance of miRNA precursors for these fast-decaying miRNAs. Using a statistical approach to the Droplet Digital PCR (ddPCR) method, we compared the absolute sum of the miRNA precursors in 5D and 5D+2L seedlings. In the tested samples, *pri-miR157* and *pri-miR158* had 57 and 46.5 copies per 0.02 ng of total RNA, respectively, *pri-miR160* and *pri-miR319* had 30.9 and 38 copies, while *pri-miR159* had a much lower expression level with 23.9 copies (Figure 7D). This result indicated that the levels of pri-miRNA could also determine the production rate of a short-lived miRNA. We also found that pri-miR163 had 827 copies, which was remarkably higher than the copy number of the other pri-miRNAs observed (Figure 7D). Moreover, the stability of miR163 could be attributed to the 192-fold increment of miR163 by light treatments (Figures 3B, 7C and 7D). These results indicated the balance between the degree of *MIR* gene expression (the level of *pri-miRNA* transcripts), microprocessor activity,

and miRNA-decay rate that determines the expression levels of miRNAs during photomorphogenesis (Table S1).

DISCUSSION

We demonstrated that the transition from skoto- to photo-morphogenic development triggers the accumulation of the elements of core microprocessor and miRNA precursors. Light immediately stabilizes DCL1, SE, and HYL1 via post-translational regulation, and simultaneously up-regulates the expression of these genes. With this two-track regulation, de-etiolated seedlings dramatically increase the levels of the core microprocessor proteins in response to white, blue, red, and far-red light (Figure 1). These observations contradict a recent study that suggests destabilization of DCL1 and HYL1 during dark to red light transition (Sun et al., 2018). We speculate that the contradiction could be caused by incognizant differences in the experimental conditions between the two studies. However, to ensure our experimental conditions, using two different types of anti-DCL1 antibodies (see Methods), we confirmed that both endogenous DCL1 and heterologous DCL1-6myc with expected molecular weights of ~215 kDa and ~250 kDa, respectively, are stabilized by light (Figures 1, S1 and S9). Despite the light-mediated accumulation of the core microprocessor components and up-regulation of pri-miRNAs, most miRNAs displayed minor fluctuations in their amounts upon skoto- to photomorphogenic transition. To reconcile this discordance, we showed that the processing activity of the core microprocessor is down-regulated by light exposure, possibly due to a light-induced suppressor. This mechanism is enough to account for minor fluctuations of miRNAs such as miR160 and miR319. However, the low processing activity of the microprocessor is insufficient to explain the other notably down-regulated miRNAs such as miR156 and miR159, particularly, considering the up-regulation of their pri-miRNA transcripts. The reduction of these miRNAs by light could be explained by accounting for another variable—miRNA turnover. Therefore, SDN1 could be concomitantly involved in the modulation of miRNAs (Figure 7). The light-increased SDN1 accounts for the diminished levels of miR156 and miR159, each of which has a relatively short half-life. In addition to these two negative regulations, the degree of *MIRNA* gene expression could be another determinant for some miRNAs, such as miR157 and miR158. As seen in the increments of their pri-miRNA transcript levels, the transcriptional up-regulation of some *MIRNA* genes could countervail both negative

389 regulations during de-etiolation (Figure 7D). In the same context, the stability of miR163 and
390 the high expression level of pri-miR163 could result in a significant increase of miR163
391 (Figures 3A, 3B and 4G). By contrast, the light-induced suppressor activity and SDN1 activity
392 could influence the production of miR159 under light because such miRNA decays rapidly and
393 the level of its pri-miRNA is relatively low (Figures 7C, 3C, 4B, and S5D). However, the levels
394 of some miRNAs are unexplained by these three regulatory notions. For instance, the notable
395 reduction of miR166 in CL seedlings as compared to 5D seedlings (Figures 4G and S6A) is
396 mysterious not only because all the *pri-miR166* transcripts accumulated (Figure 4D), but also
397 the half-life of miR166 was longer (Figure 7C). In plants, pri-miRNAs vary in size and shape,
398 and are processed by different processing modes (Moro et al., 2018). Thus, one plausible
399 speculation is that the regulation of the microprocessor's accessibility to specific pri-miRNAs
400 via co-factors (Li et al., 2017; Ren et al., 2012; Wang et al., 2019b) might be differentially
401 modulated by light, but there is no evidence to substantiate. To elucidate this puzzle, light-
402 induced reduction of specific miRNAs should be investigated to clarify if their half-life is
403 differentially-modulated during de-etiolation, day-and-night shift, shading, and continuous
404 light irradiation, and if some of the specific sequences are more susceptible to the RNases.
405 Above all, to clearly understand the miRNA-biogenetic inconsistency observed during
406 photomorphogenesis, it is essential to identify the unknown suppressor and/or possible light-
407 induced post-translational modifications of microprocessor components (e.g., possible
408 phosphorylation-mediated regulation of DCL1). CHR2 could also be a possible suppressor, as
409 its negative regulatory function on miRNA biogenesis has been demonstrated (Wang et al.,
410 2018). However, *CHR2* does not seem to be induced by light at the transcriptional level (Figure
411 S10).

412 Light provides important cues for the distinctive transcriptome shifts that define
413 photomorphogenesis. Our findings here suggest a novel mechanism, the miRNA-biogenetic
414 inconsistency, at play describing the regulatory gearshift in miRNA biogenesis during
415 photomorphogenesis. Through this mechanism, de-etiolated seedlings could modulate the
416 balance between light-induced transcriptome and miRNA-mediated gene silencing in the early
417 stages of photomorphogenesis (Figure 7E). Without such a mechanism, de-etiolated seedlings
418 could possibly experience internal conflicts between miRNAs and light-responsive
419 transcripts—most of them are transcription factors, and that eventually result in pleiotropic
420 defects in the growth and development of plants or risk seedling survivability (Figures 6H and
421 6I). The miRNA-biogenetic inconsistency could be essential for seedlings buried deep in the

soil. For such seedlings, reaching the surface with an elongated hypocotyl expends most of their endosperm. Barely emerged seedlings could improve their survivability by conditionally suppressing miRNA biogenesis to promote expression of favorable genes, which may enable energy-saving while facilitating adapting to ever-changing light conditions (Figures 6G and 6H). When the seedlings survive and receive optimal light, they could increase the expression of additional microprocessor components to increase miRNAs, as what was observed in the prolonged light-grown seedlings (Figure 4). However, to further validate this speculation, investigation of the detailed networks between miRNAs biogenesis and light signaling is warranted. We emphasize that the challenges should include at least these three questions; i) whether miRNA-biogenetic components are directly involved in the light signaling pathway, regardless of their function in miRNA biogenesis; ii) whether microprocessor components are differently regulated during the developmental transition from skoto- to photomorphogenesis, light adaptation, shade avoidance, and photoperiodic changes; and iii) how light influences the post-translational regulation of microprocessor components such as proteolysis (Cho et al., 2014), and phosphorylation (Achkar et al., 2018). Furthermore, a study recently reported intriguing relationship between pri-miRNAs and corresponding miRNAs under differential abiotic stresses that partially resemble the miRNA-biogenetic inconsistency what we described (Barciszewska-Pacak et al., 2015). Likewise, extensive post-transcriptional regulations of miRNAs implicate for cancers that also resemble the miRNA-biogenetic inconsistency (Thomson et al., 2006). These observations suggest that the miRNA-biogenetic inconsistency can be triggered by different environmental stimuli and developmental regulations thus the underlying mechanism should be further investigated focusing on the functionality of miRNAs during these responses in plants and animals.

METHODS

Plant material and growth conditions

Arabidopsis plants in the Columbia background were used. The *cry1-304/cry2-1, phyA-211/phyB-9* and *35S:miP-DCL1* transgenic lines were obtained from previous studies (Dolde et al., 2018; Lascève et al., 1999; Reed et al., 1994). The *35S:DCL1-6Myc* and *35S:2B8-DCL1-2xFlag* constructs were introduced in plants by *Agrobacterium tumefaciens* (GV3101 strain)-mediated infiltration using the floral dip method. Arabidopsis seeds were surface-sterilized and plated onto half-strength Murashige & Skoog (MS) solid media including 1% sucrose

and 2 mM MES, pH 5.7. After plating, the seeds were stratified for 3 days at 4 °C, and then exposed to 6 h of white fluorescent light (100 μmol m^{-2} s^{-1}) at 22 °C to initiate germination. After this, the plates were wrapped in three layers of aluminum foil and incubated at 22 °C for further five days. Subsequently, irradiation with blue (470 nm, 10 μmol m^{-2} s^{-1}), red (660 nm, 10 μmol m^{-2} s^{-1}) and far-red (740 nm, 10 μmol m^{-2} s^{-1}) light-emitting diodes (LEDs) or white light at 22 °C was performed for the indicated time periods.

RNA extraction and analyses

Total RNA was extracted from Arabidopsis seedlings using RNeasy Plant Mini kit (Qiagen). Reverse transcription was performed using iScript™ cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. Primers for pri-miRNAs, *HYL1*, *SERRATE*, *DCL1*, and *UBQ10* are listed in Supplementary Table S1. qRT–PCR was carried out using Thermo Scientific™ PikoReal™ Real-Time PCR System. Relative amounts of transcripts were obtained by calibrating threshold cycles of target genes with that of UBQ10 reference gene. Calculations were performed using the formula $2^{(-\Delta\Delta C_T)}$, where C_T is the cycle number at which the fluorescence reaches the threshold point for detection. The experiments were performed with three independent biological replicates.

Small RNA isolation and northern blot analysis

Total RNA was isolated from etiolated or de-etiolated seedlings using TRIzol reagent (Invitrogen). The extracted aqueous phase was precipitated with 2-propanol twice (100 and 75%) and dissolved in 50% formamide. Purified RNA was resolved using 5-15% denaturing polyacrylamide gel (National Diagnostics) before transferring to a nylon membrane (Amersham). The 5′-end-labelled DNA probes were used for hybridization of membrane blots for 12 h (Ambion). Blots were washed twice with washing buffer (2× SSC and 0.1% SDS) for 20 min each. Hybridization signals were detected using a phosphor-imager scanner (BAS-2500, Fujifilm).

Deep sequencing and analysis of small RNAs

We performed two independent sets of miRNA sequencing experiments using the Illumina platforms of 5D seedlings, 5D+1L seedlings, and 5D+3L seedlings: one was performed in Korea (Set-1), and another one in Denmark (Set-2). We obtained 42, 42, and 48 million clean reads for set-1 and 39, 42, and 46 million clean reads for set-2 that were aligned with

the Arabidopsis genome. We compared the normalized counts of mature miRNAs in 5D+1L seedlings and 5D+3L seedlings versus 5D seedlings. Construction of small RNA libraries with 5D, 5D+1L, and 5D+3L samples, deep sequencing, and analysis of small RNAs were performed by Macrogen (Belgium) or LAS Inc. (Korea). The expression levels of miRNAs (transcripts per 10 million, TPTM) in the indicated samples were calculated by normalizing the miRNA counts with the total number of clean reads in the small RNA libraries. Furthermore, a part of the analysis was additionally tested in Argentina (Figure S3F, Dataset S3).

Dark to light transition assay

For the dark to light transient assay, etiolated five-day-old wild-type, *cry1-304/cry2-1, phyA-211phy/B-9* and Col-0/*35S:DCL1-6Myc* transgenic seedlings were transferred to continuous blue, red, and far-red LED illumination boxes or a white light growth chamber at 22°C for the indicated time periods. Samples were harvested under safe green LED light in a dark room.

Production of antibodies

We generated rabbit polyclonal α-DCL1 antibody using GST-DCL1-N-terminal fragment as an antigen and mouse polyclonal α-DCL1 antibody using synthetic oligopeptides that match the DUF283 domain of DCL1. Specific α-DCL1 antibodies were purified from rabbit and mouse serum using protein A agarose resin.

Protein gel blot analyses

A frozen powder of the samples (100 mg) was directly mixed with 5 × SDS sample buffer for 10 min, and 10 μg of total proteins were resolved using 8-12% SDS-PAGE after boiling at 100°C. The proteins were transferred to a PVDF membrane. Blots were hybridized with α-DCL1 antibody (dilution 1:3,000, this study), α-SERRATE antibody (dilution 1:5,000, Cho et al., 2014), α-HYL1 antibody (dilution 1:20,000, Yang et al., 2010), α-ACTIN antibody (dilution 1:3,000, Agrisera, AS13 2640), α-Histone H3 antibody (dilution 1:10,000, Agrisera AS10 710), α-2B8 epitope antibody (dilution 1:10,000, S.-H. Bhoo provided), and α-SDN1 antibody (dilution 1:1000, Abmart, X-A3KPE8 –C) respectively.

***In vitro* transcription of RNA**

RNA substrates were *in vitro* transcribed under the T7 promoter using PCR-generated

templates. The templates and primers used for PCRs and the synthetic pri-miRNAs are as listed in a previous study (Zhu et al., 2013). The *in vitro* transcription of RNAs was carried out in a 20-μL reaction incubated at 37 °C for 2 h or at 30 °C overnight as follows: DNA template (200 ng), 4 μL of 5× transcription buffer (400 mM HEPES, pH 7.5, 10 mM spermidine, 200 mM DTT, 125 mM $MgCl_2$ and 20 mM of each NTP), 1 μL of RNase inhibitor (Ambion), 2 μL of T7 RNA polymerase and up to 20 μL of water. RNA was fractionated on 5% polyacrylamide and 6 M urea gel (denaturing gel), and eluted using a nucleotide removal kit (Qiagen, Cat# 28304). For internal labeling, [α-^{32}P]-UTP (PerkinElmer) was included in the NTP mixture (20 mM CTP, 20 mM ATP, 20 mM GTP and 4 mM UTP) for *in vitro* transcription as described above. All the labeled RNAs were resolved using 5% denaturing gel and eluted from the resolved gel slice. Labeled RNAs were folded by heating to 95 °C for 2 min, slowly cooled to room temperature and normalized to ~2 × 103 c.p.m. μL^{-1}.

Immunoprecipitation and *in vitro* pri-miRNA processing assay

For the *in vitro* experiment processing assay, the core microprocessor complex was purified using α-Myc antibody from five-day-old etiolated and two-day-old de-etiolated Col-0/35S:DCL1-6Myc seedlings. Immunoprecipitation was performed as described (Zhu et al., 2011) with some modifications. Arabidopsis samples were ground in liquid nitrogen, and protein-sRNA complexes were extracted using immunoprecipitation buffer (40 mM Tris-HCl, pH 7.5, 300 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 0.2 mM EDTA, pH 8, 0.2% Triton X-100, 1 mM PMSF, 2% glycerol and 0.3% (vol/vol) proteinase inhibitor cocktail (Sigma)) and 1 tablet of EDTA-free protease inhibitor cocktail (Roche) per 25 mL immunoprecipitation buffer. After removal of insoluble materials by centrifugation twice at 15000 ×*g* for 15 min at 4 °C, extracts were incubated with α-Myc Antibody (GeneScript, A00704) for 3 h at 4 °C. The mixtures were incubated for 3 h at 4 °C with SureBeads™ Protein A Magnetic Beads (BioRad, #1614013). The beads were washed three times with immunoprecipitation buffer and then three times with washing buffer (20 mM Tris-HCl, pH 7.5, 1 mM DTT, 4 mM $MgCl_2$ and 100 mM KCl). Briefly, *in vitro* DCL1 cleavage assays were performed in a total volume of 30 μl in 20 mM Tris-HCl, pH 7.5, 53 mM KCl, 4 mM $MgCl_2$, 1 mM DTT, 7.5 mM ATP, 1 mM GTP and 1 μl^{-1} RNase Inhibitor (Ambion), including 1 μl of [α-32P]-UTP labeled RNA (~2 × 103 c.p.m.) and 15 μL of the immunopurified magnetic beads in washing buffer. The reaction mixture was incubated at 37 °C for 0.5-3 h. After extraction with phenol-chloroform and ethanol, the processing products were fractionated using 5-15% denaturing gels. The RNA marker used was

synthesized miR166 that was 5' end-labeled according to the manufacturer's protocol with [α-^{32}P]-ATP (PerkinElmer). The denaturing gel was dried in a Gel Dryer (Bio-Rad) at 65 °C for 2 h. The processed RNA products were detected after exposure overnight to a phosphor plate and signals were detected with a phosphorimager scanner (BAS-2500, Fujifilm)

Amanitin-chase assay

5D and 5D+2L seedlings were mock-treated or incubated with 10 mM of α-amanitin (Sigma, A2223) for 6 h. Total RNA was isolated using TRIzol reagent (Invitrogen) and was used for northern blot analyses.

Droplet Digital PCR

The QX200™ Droplet Digital™ PCR System (Bio-Rad) was used in this study according to the manufacturer's instructions. Droplets were generated by a Droplet Generator (DG). The prepared droplets were transferred to a 96-well PCR plate. The PCR plate was subsequently heat-sealed and amplified in a C1000 Touch™ deep-well thermal cycler (Bio-Rad). The thermocycling protocol was: initial denaturation at 95 °C for 5 min, then 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s (temperature ramp 2 °C/s) and, final incubation at 98 °C for 10 min and storage at 4 °C. After cycling, the 96-well plate was fixed to a plate holder and placed in the Droplet Reader (Bio-Rad). Primers used for Droplet Digital PCR analyses are listed in the Supplementary Table 2.

Author contributions

SWY, SKC, FN and AV conceived this study, wrote the manuscripts, critically reviewed. AM, SW, and PM edited; SKC, SWC, HJJ, GMK, and AV performed most of the molecular and biochemical analyses; MYR, ALA, NPA and PM conducted the informatics activity; SWC, SW, and UD conducted the phenotype analyses of transgenic plants.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2017R1A2B4010255 and 2018R1A6A1A03025607); NRF-2017R1A6A3A11035981 to MYR; and NRF-2017H1A2A1044209 to SWC. The research was

supported in part by Brain Korea 21(BK21) PLUS program / SWC and GMK are fellowship awardee by BK21 PLUS program. The research in Hungary was supported by grants from the Economic Development and Innovation Operative Program (GINOP-2.3.2-15-2016-00015 and GINOP-2.3.2-15-2016-00032). We thanks to A. Maizel and S.-H. Bhoo for *pMIR390a:GUS* transgenic plants and 2B8 antibody, respectively.

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775

776 Figure legends

777

778 Figure 1. Light augments the core microprocessor components DCL1, SE, and HYL1

779 **A.** Expression levels of the core microprocessor components in five-day-old etiolated seedlings
780 (5D) were increased by white or blue light treatments. **B.** Expression levels of the core
781 microprocessor components in 5D seedlings were increased by white or red light treatments.
782 **C.** Expression levels of the core microprocessor components in 5D seedlings were increased
783 by white or far-red light treatments. **D.** Expression levels of the core microprocessor
784 components in four-day-old etiolated seedlings were increased by blue, red, or far-red (FR)
785 irradiation for 3 h and 5 h. **E.** Expression levels of DCL1 and HYL1 in 2-week-old light-grown
786 plantlets (L) were diminished after 12 h darkness (D). **F.** qRT-PCR analysis revealed that
787 transcripts of the core microprocessor components increased slightly after two days of light
788 treatment. Data are plotted as the average value of four biological replicates, error bars
789 indicate ±s.d. (n=12). *UBQ10* transcript level was used as the calibration reference. **G.**
790 Effects of proteolysis inhibitors on the core microprocessor components. Four-day-old
791 etiolated seedlings were treated with Mock, MG132 (10 μM), and PIs (0.2×) for 2 h or 6 h.
792 In panels A-C: seedlings were grown in darkness for 5-8 days (5D-8D). 5D seedlings were

irradiated with white or different monochromatic light for 1-3 days (5D+1L, 5D+2L, 5D+3L). In panels A-E and G, the levels of endogenous DCL1, SE, and HYL1 were determined with α-DCL1 (Agrisera), α-DCL1-N (mouse polyclonal), α-DCL1-Rb (rabbit polyclonal), α-SE, or α-HYL1 antibodies, respectively. Uniform loading of samples was confirmed with α-actin or/and α-histone antibodies.

Figure 2. Photoreceptors are important for mediating the light-induced accumulation of the core microprocessor components.

A. Expression levels of the core microprocessor components in *phyA/phyB* double mutant background under white light. **B.** Expression levels of the core microprocessor components in *cry1/cry2* double mutant background under white light. **C.** Expression levels of the core microprocessor components in *phyA/phyB* double mutant background under red light. **D.** Expression levels of the core microprocessor components in *phyA/phyB* double mutant background under far-red light. **E.** Expression levels of the core microprocessor components in *cry1/cry2* double mutant background under blue light. In panels a-e: Seedlings were grown in darkness for 5-8 days (5D-8D). Five-day-old etiolated (5D) seedlings were irradiated with white or different monochromatic light for 1-3 days (5D+1L, 5D+2L, 5D+3L). Plants were also grown in constant light for 17 days (CL). **F**. Expression levels of the core microprocessor components in Col-0, *phyA/phyB*, and *cry1/cry2* mutants were grown for the indicated period with the corresponding light treatment. In all panels, the levels of endogenous DCL1, SE, and HYL1 were determined using α-DCL1, α-SE, or α-HYL1 antibodies, respectively. Uniform loading of samples was confirmed with α-actin or/and α-histone antibodies.

Figure 3. Primary miRNAs and small RNA sequencing analysis in five-day old etiolated (5D) seedlings and de-etiolated seedlings treated with white-light for 1 and 3 days.

A. qRT–PCR analysis of *pri-miRNA* transcripts in 5-day-old etiolated seedlings (5D) that were irradiated with white light for 1 day (5D+1L) 2 days (5D+2L) or 3 days (5D+3L). Data are plotted as the average value of four biological replicates, error bars indicate ±s.d. (n=12). *UBQ10* transcripts were used as a calibration reference. **B.** Highly up-regulated miRNAs with reading frequencies >100. 17% of miRNAs increased with change-ratio >0.5 ($\log_2$DTPTM > 0.5). In panels A-E: The results of two independent small RNA sequencing experiments (SET1 and SET2) are presented in this figure. **C.** Highly down-regulated

826 miRNAs with reading frequencies >100. 5% of miRNAs decreased with change-ratio <-0.5
827 (log_2DTPTM < - 0.5). **D.** Northern blot analysis of selected up- and down-regulated miRNAs
828 upon exposure to white light. The loading control used is 5.8S rRNA.
829
830 **Figure 4. Accumulation of core microprocessor components and pri-miRNAs under**
831 **prolonged light conditions**
832 **A-E.** qRT-PCR analysis of *pri-miRNA* transcripts in etiolated and de-etiolated seedlings
833 irradiated with white light for 3 (5D+3L) or 12 (5d+12L) days. CL: 17-day-old light-grown
834 plants. The relative amount of pri-miRNAs was calculated with ±s.d. from three biological
835 repeats (*P < 0.05; **P < 0.005; unpaired, two-tailed Student's *t*-test). **F.** Northern blot
836 analysis of selected up- and down-regulated mature miRNAs in etiolated and de-etiolated
837 seedlings under extended light treatments. Total RNA samples were resolved by gradient-
838 PAGE (5-15%) and hybridized with specific radioisotope labeled probes. 5.8S rRNA is used
839 as loading control, CL: 17-day-old light-grown plants. **G.** Comparison of the expression of
840 tested miRNAs from two independent small RNA sequencing experiments (SET 1 and SET
841 2). **H.** qRT–PCR analysis of *DCL1*, *SE*, and *HYL1* transcripts in 5D seedlings subjected to
842 prolonged light conditions (1-12 days) and in plants grown for 17 days in constant light (CL)
843 seedlings. In all the qRT-PCR analyses, data plotted is average of four biological replicates.
844 Error bars indicate ±s.d. (n=14). *UBQ10* transcripts were used as the calibration reference.
845 **I.** The protein levels of DCL1, SE, and HYL1 in etiolated seedlings under prolonged light
846 conditions are shown. The protein level of actin is presented as the loading control. RuBisCo
847 is used as a control for light-growth period.
848
849 **Figure 5. Deceleration of core microprocessor processing activity during transition**
850 **from skoto- to photomorphogenic development**
851 **A.** Schematic depicting the use of artificial $^{32}\alpha$-UTP incorporated pri-mR166c substrate for
852 the *in vitro* enzymatic assay. **B.** *In vitro* pri-miRNA processing assay. Immunoprecipitated
853 DCL1-6Myc isolated from five-day-old etiolated (5D) Col-0 seedlings, 5D Col-
854 0/*35S:DCL1-6Myc* seedlings, and 5D Col-0/*35S:DCL1-6Myc* seedlings irradiated with white
855 light for one-day (5D+1L) were incubated with $^{32}\alpha$-UTP incorporated pri-mR166c substrate
856 for three hours. Red arrowheads indicate the processed fragments. The red asterisk marks a
857 band corresponding to mature miRNA size. **C**. *In vitro* pri-miRNA processing assay. Cold
858 pri-miR166c substrates were incubated with immunoprecipitated DCL1-6Myc isolated from

Col-0/*35S:DCL1-6Myc* seedlings grown under 5D or 5D+1L light regimes. PAGE-resolved cleavage products were blotted with $^{32}\gamma$-ATP-labeled miR166 probe or non-miR166 probe. The lane marked as SM contains non-radioactive synthetic 22-nt miR166 as a positive control**. D.** *In vitro* pri-miRNA processing assay. Upper panel shows the levels of immunoprecipitated DCL1-6Myc isolated from 5D and 5D+1L Col-0/*35S:DCL1-6Myc* seedlings. **E.** *In vitro* pri-miRNA processing assay. Upper panel shows the immunoprecipitated DCL1-6Myc adjusted to an equal level, isolated from isolated from 5D and 5D+1L Col-0/*35S:DCL1-6Myc* seedlings. Bottom panels of panels **D** and **E** show the cleaved pri-miR166c intermediates and mature miR166 from *in vitro* pri-miRNA processing assay. The asterisk denotes the processed miR166 from the artificial pri-miR166c substrate. SM means size maker of $^{32-\gamma}$ATP-labeled synthetic 22-nt miR166. Red arrowhead indicates the processed fragments.

Figure 6. Overexpression of DCL1-PAZ domain as a miR-DCL1 in Col-0 increases the functionality of microprocessor

A. Schematic drawing of the microProtein(miP)-DCL1 system (Dolde et al., 2018). **B.** Tested miRNAs were up-regulated in 17-day-old light-grown (CL) miP-DCL1 over-expressing transgenic plantlets as compared to that in the wild-type plantlets. **C**. qRT-PCR analysis of *pri-miRNA* transcripts in CL Col-0 and CL miP-DCL1 over-expressing transgenic plantlets. The relative amount of pri-miRNAs was calculated with ±s.d. from three biological repeats (*$P < 0.05$; **$P < 0.005$; unpaired, two-tailed Student's *t*-test). **D-F**. Northern blot analyses show restoration of light-induced reduction of microprocessor activity in the miP-DCP1 system. Overexpression of miP-DCP1 maintains or further increases miRNA production in 5D seedlings and 5D seedlings that were irradiated for 1, 2 or 3 days (5D+1L, 5D+2L, 5D+3L) with white light. The loading control was 5.8S rRNA. **G.** 5D+1L seedlings of miP-DCL1 grown in sugar-free MS medium showing proportional reduction in hypocotyl opening. **H.** 6D+5L seedling of miP-DCL1 transgenic plants grown in sugar-free MS medium with a significantly low survival rate during photomorphogenesis. The data are average of six biological samples with ±s.d. (n=180). **I.** Two-week-old plants expression miP-DCL1 show slight defects in rosette development.

Figure 7. Light induces the accumulation of *SDN1* transcripts and proteins

A. qRT-PCR analysis of *SDN1, SDN2,* and *SDN3* transcripts in etiolated seedlings (5 to 8-

892 days old) or in five-day old etiolated (5D) seedlings irradiated with white light for 1-3 days
893 (5D+1L - 5D+3L). The data are average of four biological samples with ±s.d. (*n*=12).
894 *UBQ10* transcripts were used as the calibration reference. **B.** Expression profile of SDN1
895 protein in etiolated seedlings (5 to 8-days old) or in 5D seedlings irradiated with white light
896 for 1-3 days (5D+1L - 5D+3L). The levels of endogenous SDN1 were determined with α-
897 SDN1 antibody. In all tests, uniform sample loading was ascertained with α-actin and α-
898 histone antibodies. **C.** Amanitin-chase assay. 5D+2L seedlings and 5D seedlings were
899 treated with α-amanitin (10 mM) for 6 h to block *MIR* gene transcription and miRNA decay
900 monitored (upper panel). Levels of miRNA were determined by northern blot analysis. The
901 loading control was 5.8S rRNA (lower panel). Red asterisks denote rapidly-decaying miRNAs.
902 **D.** The quantification of pri-miRNA levels in 5D+2L seedlings based on droplet digital PCR
903 analysis. The ordinate scales indicate the fluorescent amplitude. Pink line indicates the
904 threshold, above which, blue dots are positive droplets containing at least one copy of the target
905 cDNA, and below which, gray dots indicate negative droplets without the target cDNA (upper
906 panel). The bar graph shows the total read counts of each pri-miRNA in the 5D+2L sample
907 (lower panel). The data are values of the PoissonConfMax/Min that normalize for the
908 high/low error bar of the droplet Poisson distribution for the 95% confidence interval. **E.**
909 Graphical abstract of the miRNA-biogenetic inconsistency